DISCUSSION
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Diabetes has become the commonest non communicable disease worldwide with India as its capital and highest number of patients i.e 32 million at present and exponential growth projection of 80 million diabetics by year 2030.\(^1\) Diabetes has become one of the major causes of premature illness and death in many countries, mainly through the increased risk of cardiovascular disease (CVD). Asian Indians are more prone to type II DM at a younger age and premature cardiovascular disease.\(^5\)

Increasing urbanization and industrialization are the chief reasons for rapid increase in prevalence of type II DM.\(^55,57\) Diabetes in urban Indians is reaching epidemic scale.\(^59-65\) The prevalence of type II DM in Asian Indians ranges from 2.7 percent in rural areas to 14 percent in urban areas and up to 16-22 percent in migrant Indians living in Europe, USA, Africa and Fiji.\(^66-68\) Diabetes is the leading cause of death, disability and economic loss worldwide.\(^2\) Two major concerns are that much of the increase in diabetes will occur in developing countries due to, population growth, ageing, unhealthy diets, obesity and sedentary life styles, and that there is a growing incidence of type II DM - which accounts for 90% of all cases at a younger age.

The increasing incidence & prevalence of diabetes is putting a lot of financial, social & health care burden with decreasing productivity of Indian population. Diabetes mellitus being a multi factorial disease, total prevention is impossible. Understanding the patho-physiology of this major silent killer and its association with various measurable biochemical parameters may provide further insight in development and progression of diabetes. Various parameters have been studied in context to etiopathogenesis, pathophysiology and progression of diabetes by various researchers, focusing on biochemical parameters, lipids, inflammatory
markers and oxidative stress in India and worldwide. Most of the studies focus on a particular group of biochemical markers with small number of cases or large studies with specific one or two markers. **Very few studies comprising markers from different groups with sizable number of type 2 DM patients with and without microangiopathy have been carried out especially in context of Indian patients.** This study was taken up with the aim to compare biochemical parameters of various groups in the same set of type 2 DM patients so as to establish their role and inter relations with each other.

As shown in Table 2, Figure 18 & 19 Total 484 individuals participated in the study, 165 (34.09%) were normal forming the Control Group A, 162 (33.47%) were Diabetics without Nephropathy i.e. having no albuminuria forming Group B-T2DM and 157 (32.44%) were Diabetics with Nephropathy i.e. having albuminuria forming Group C-T2DMN. This Group C was further divided as those having Microalbuminuria (C I, N=127) and Macroalbuminuria (C II, N=30). The total number of males in the study was 263 (54.34%) and total number of females was 221 (45.66%). The male to female ratio was comparable in Group A (0.99) and Group B (1.08) (Table 2 and Figure 20). **Similar near equal incidence of type 2 DM in males and females have been reported in population based studies** like NUDS\(^{61}\) and PODIS\(^{64}\) in India. While Singh TP et al\(^{78}\) and Sarah Wild et al\(^{1}\) reported high incidence of type 2 DM in males compared to females. In Group C male to female ratio was higher (1.62), showing that **more number of males had Diabetic Nephropathy as compared to females.** Similar findings have been reported by Bahman P et al\(^{538}\), Stratton IM et al (UKPDS)\(^{154}\) and Mogensen et al\(^{502}\) who followed type 2 DM patients for development of nephropathy. They reported high triglycerides, HbA\(_{1c}\), hypertension, smoking and duration of diabetes as predictors of progression to diabetic nephropathy.
The mean Age was comparable in the three groups. Mean age in the healthy controls (Group A) was 54.35 ± 11.00 yrs, Diabetics without Nephropathy (Group B – T2DM) was 53.18 ± 10.40 yrs and Diabetics with Nephropathy (Group C – T2DMN) was 54.59 ± 7.85 yrs (Table 3 and Figure 22). The study population was also divided on the basis of Age into 3 groups (Figure 21) and there was a predominance of subjects in the Age Group 41-60 yrs. Maximum prevalence of Diabetes (69.28%, 221 of 319) was seen in this age group as compared to healthy controls (62.42%, 103 of 165). The highest rate of incidence of type 2 DM in the age group of 40 to 60 years in present study is in accordance with the global (King H et al3, Sarah Wild et al4) and Indian studies (NUDS61, PODIS64, V Mohan et al5). As per study by Sarah Wild et al1 in developing countries, the majority of people with diabetes are in the 45- to 64-year age range, in contrast, the majority of people with diabetes in developed countries are above 64 years of age. The age structure of the world total follows the trend for developing countries. Over the past 30 yr, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people.5 This is a disturbing finding as the earlier age of onset combined with increasing prevalence of diabetes could have adverse effects on nation’s health and economy.

The duration from diagnosis of Diabetes in Group B (T2DM) & Group C (T2DMN) was 5.00 ± 2.20 yrs & 6.55 ± 2.71yrs respectively. Thus the diabetics with nephropathy had significantly longer duration of disease compared to diabetics without nephropathy. (Table 3 and Figure 23) This finding is supported by the prospective studies of type 2 DM patients for development of DN by Bahman P et al538, Stratton IM et al (UKPDS)154 and Mogensen et al.502
GLYCEMIC STATUS IN DIABETES

Though the latest recommendations by WHO regarding use of HbA1c as diagnostic parameters for diabetes have been proposed\(^{102}\), fasting blood glucose and post glucose/post prandial blood glucose still remain the main stem for diagnosing DM. HbA1c is the most commonly measured method of assessing chronic glycemia in clinical practice.\(^{89}\) It has been used as a surrogate marker of long term glycemic control in patients with DM, both in clinical practice and in countless studies and trials.\(^{90}\) The value of the HbA1c (which are expressed as a percentage of total Hemoglobin) gives a time-weighted indication of the average glucose over the lifespan of the red cell.\(^{92}\)

In present study as per table 4 and 5 the mean values of fasting & post prandial glucose in diabetics were group B (T2DM) 152.05 ± 57.90 mg/dl & 239.25 ± 97.02 mg/dl, group C (T2DMN) 177.50 ± 64.79 mg/dl & 287.49 ± 116.91 mg/dl as compared to control (Group A) 85.97 ± 9.57 mg/dl & 114.56 ± 13.59 mg/dl. The mean fasting and post prandial plasma glucose values in diabetics with and without nephropathy were significantly higher compared to control group (P<0.001). The findings regarding glucose levels in present study support the theory of insulin resistance leading to hyperglycemia in type 2 DM and also fulfill the criteria of plasma glucose for diagnosing diabetes.\(^{53,58,83}\)

The mean values of HbA1c & eAG in diabetics were group B (T2DM) 8.46 ± 1.56% & 197.40 ± 46.78 mg/dl, group C (T2DMN) 9.21 ± 1.84% & 218.25 ± 54.65mg/dl as compared to group A (control) 5.73 ± 0.35% 117.14 ± 9.86mg/dl (table 4). HbA1c mean values in diabetics (group B & C) were significantly higher with higher eAG levels compared to the control (group A) values. Higher values of glycosylated hemoglobin in diabetics have been reported by ADA\(^{501}\), Ikuo Yokoyama et al\(^{8}\), Shehnaz A Sheikh et al\(^{11}\), Goldstein DE et al\(^{89}\), Jeffcoate SL et al\(^{90}\), Gabbay KH et al\(^{96}\) and others. In normoglycemic subjects, a small proportion of HbA is
attached to carbohydrate moiety and forms HbAIC. In conditions of sustained hyperglycemia, such as DM the proportion of Hb that is glycosylated is increased substantially. This glycosylation is a result of post translational modification of HbA molecules. The binding of glucose is a non enzymatic process that occurs continuously during the life of RBC.102 The relationship between HbAIC and chronic glycemia has been explored in several studies that have supported the association of HbAIC with average glucose levels over the preceding 5–12 weeks530 and the average glucose levels were found to be significantly higher in diabetics compared to non diabetics.530, 102

The mean values of fasting and post prandial plasma glucose, HbAIC and eAG were significantly higher in diabetics with nephropathy (group C) compared to diabetics without nephropathy (group B) (P<0.001) as shown in table 5. Similar higher values of glucose and HbAIC have been reported in diabetics with microangiopathy, retinopathy and nephropathy compared to the diabetics without microangiopathy by Ikuo Yokoyama et al, Shehnaz A Sheikh et al11, J Chen et al24, Jeffcoate SL et al90, Colagiuri S C et al101 and Tapp RJ et al.107 For all three measures of glycemia, the value above which the prevalence of microangiopathy begins to rise rapidly has differed to some extent between studies. Persistent elevations in blood glucose (and, therefore, HbAIC) increase the risk for the long-term vascular complications of diabetes such as coronary disease, heart attack, stroke, heart failure, kidney failure, blindness, erectile dysfunction, neuropathy, gangrene, and gastroparesis. Various studies by S Roy et al6, H Kusunoki et al7, Shehnaz A Sheikh et al11, Jeffcoate SL et al90, P. Marchetti et al91, and Syed Muhammad Shahid et al123 have put forth different molecular mechanisms of endothelial cell damage in microvasculature via polyol pathway, sorbitol pathway, advanced end glycation products and increased free radicals in sustained hyperglycemia state of type 2 DM leading to microangiopathy. Tight glycemic control has been shown to positively influence the risk
outcomes in diabetic nephropathy. On a cellular level, it has been known to reverse glomerular hypertrophy and hyperfiltration, both important mechanisms for early glomerular injury. Long term prospective studies are required in all major ethnic groups to establish more precisely the glucose and HbA1c levels predictive of microvascular and macrovascular complications.

As per Table 6 the mean values of fasting & post prandial glucose in diabetics with microalbuminuria (Group C I) were 170.92 ± 61.96 mg/dl & 277.75 ± 115.32 mg/dl & in diabetics with macroalbuminuria (Group C II) were 205.33 ± 70.06 mg/dl & 328.73 ± 116.47 mg/dl. Whereas the mean values of HbA1c & eAG in diabetics with microalbuminuria (Group C I) was 9.09% ± 1.79 & 214.47 ± 52.10 mg/dl & in diabetics with macroalbuminuria (Group C II) was 9.71 ± 1.99 & 234.22 ± 62.84 mg/dl respectively. This data shows that the mean values of fasting & post prandial glucose in diabetics with macroalbuminuria were high as compared to diabetics with microalbuminuria & the difference was significant statistically (p ≤ .05). Whereas the mean values of HbA1c & eAG in diabetics with microalbuminuria & those with macroalbuminuria when compared statistically, the differences were not significant. The majority of studies evaluating the relation between glycated hemoglobin and the risk of nephropathy have evaluated the risk of developing microalbuminuria. Studies by Shehnaz A Sheikh et al, Jeffcoate SL et al, K. Murugan et al, Stratton IM et al, and Mogensen CE et al show a strong and significant relation between glycated hemoglobin and the risk of microalbuminuria in individuals with type 1 and type 2 DM. Although fewer data exist on the relation between glycated hemoglobin and risk of macroalbuminuria and on the relation between glycated hemoglobin and the risk of nephropathy progression, several cohort studies and clinical trials support a strong and significant positive association in individuals with type 1 and type 2 DM. HbA1c levels have been shown to be independent predictor of nephropathy in type II DM.
with the highest HbA1c values in macroalbuminuria group of type II diabetics in studies by Christa Meisinger et al.\textsuperscript{119} and V Mohan et al.\textsuperscript{121}

Table 7 show the mean values of fasting glucose in male & female subjects of group B (T2DM) 154.37 ± 52.83 mg/dl, 149.55 ± 63.16 mg/dl & group C (T2DMN) 175.31 ± 59.46 mg/dl, 181.03 ± 72.97 mg/dl as compared to group A (control) 86.67 ± 9.84 mg/dl, 85.28 ± 9.31 mg/dl respectively. The mean post prandial values in male & female subjects of group B (T2DM) 245.07 ± 96.78 mg/dl, 232.99 ± 97.51 mg/dl & group C (T2DMN) 279.71 ± 118.00 mg/dl, 300.07 ± 114.99 mg/dl as compared to control Group A 113.48 ± 14.39 mg/dl, 115.64 ± 12.76 mg/dl respectively.

When the mean values of fasting and post prandial glucose were compared statistically among the male subjects in Group A & B, Group A & C highly significant (p < .001) difference was observed and when Group B & C were compared statistically significant (p < .05) difference was observed (table 9). The mean values of fasting and post prandial glucose when compared statistically among the female subjects also showed a similar trend (table 10). Various studies by Gabbay KH et al.\textsuperscript{96}, O'Dea K et al.\textsuperscript{53}, Ramachandran A et al.\textsuperscript{59}, Gupta A et al.\textsuperscript{60} and Misra A et al.\textsuperscript{63} have put forth similar findings in gender based studies of hyperglycemia in type 2 DM patients compared to control group with higher glucose values in diabetics with microvascular and macrovascular complications. The mean values of fasting and post prandial glucose when compared statistically among both the male & female subjects in each group showed no statistically significant difference (p > .05) (table 8). The mean values of HbA1c & eAG in male & female subjects of group B (T2DM) were 8.48 ± 1.43%, 8.44 ± 1.70% & 197.64 ± 41.95mg/dl, 197.15 ± 51.76 mg/dl respectively & group C (T2DMN) 9.23 ± 1.86%, 9.18 ± 1.81% & 218.77 ± 54.54 mg/dl, 217.41 ± 55.30 mg/dl as compared to control (group A) 5.74 ± 0.34%, 5.72 ± 0.36% & 117.54 ± 9.66 mg/dl, 116.75 ± 10.10 mg/dl respectively (table 7). When the mean values of HbA1c and eAG were compared statistically among the male subjects in group A & B, group A & C highly significant
(p < .001) difference was observed and when group B & C were compared statistically significant (p ≤ .05) difference was observed (table 9). The mean values of HbA1c and eAG when compared statistically among the female subjects also showed a similar trend (table 10). Nathan DM et al530, Momin M Gabir et al540 and IEC report100 have reported similar findings of higher HbA1c in gender based studies in type II DM. The mean values of HbA1c and eAG when compared statistically among both male & female subjects in each Group showed no statistically significant difference (p > .05) (table 8). Thus there was no gender bias in glucose, HbA1c and eAG values in controls and the diabetics with and without nephropathy. This is supported by the uniform normal range for HbA1c, fasting and post prandial glucose among males and females with universal cutoff values for diagnosis of DM irrespective of sex and age.58,82,86

**Table 11** show the mean values of fasting glucose in the age group 21-40 yrs, 41-60 yrs and 61-80 yrs of the subjects of group B (T2DM) 160.88 ± 94.93 mg/dl, 152.84 ± 57.03 mg/dl, 145.51 ± 36.02 mg/dl & group C (T2DMN) 168.11 ± 70.73 mg/dl, 186.66 ± 64.47 mg/dl, 153.18 ± 59.10 mg/dl as compared to group A (control) 87.67 ± 7.64 mg/dl, 87.17 ± 9.29 mg/dl, 82.48 ± 10.24 mg/dl respectively. The mean values of post prandial glucose in the age group 21-40 yrs, 41-60 yrs and 61-80 yrs of the subjects of group B (T2DM) were 235.97 ± 124.14 mg/dl, 226.13 ± 105.04 mg/dl, 218.97 ± 38.66 mg/dl & group C (T2DMN) were 346.78 ± 225.55 mg/dl, 300.15 ± 111.25 mg/dl, 236.79 ± 78.70 mg/dl as compared to group A (control) 118.89 ± 11.14 mg/dl, 115.44 ± 12.55 mg/dl, 110.75 ± 16.04 mg/dl respectively. The mean values of HbA1c in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 8.58 ± 1.77 %, 8.46 ± 1.60 %, 8.38 ± 1.38 % & group C (T2DMN) were 9.51 ± 2.25 %, 9.28 ± 1.88 %, 8.90 ± 1.61 % as compared to control group A 5.75 ± 0.42 %, 5.71 ± 0.36 %, 5.76 ± 0.32 % respectively. The mean values of estimated Average Glucose (eAG) in the age group 21-40 yrs, 41-60 yrs,
61-80 yrs of the subjects of group B (T2DM) were 199.75 ± 50.96 mg/dl, 197.94 ± 48.41 mg/dl, 194.62 ± 40.28 mg/dl & Group C (T2DMN) were 226.43 ± 64.83 mg/dl, 219.83 ± 56.56 mg/dl, 208.43 ± 45.60 mg/dl as compared to control group A 117.83 ± 11.94 mg/dl, 116.79 ± 10.0 mg/dl, 117.69 ± 8.75 mg/dl respectively.

When the mean values of parameters representing glycemic status were compared statistically among the age group 21-40 yrs in group A & B, group A & C significant (p ≤ .05) difference was observed except for HbA1c & eAG which were highly significant (p < .001) and when these parameters were compared in group B & C statistically non significant (p > .05) difference was observed (table 15). On comparing the mean values of parameters representing glycemic status among the age group 41-60 yrs in group A & B, group A & C and group B & C highly significant (p < .001) difference was observed except for eAG when compared between group B & C significant (p ≤ .05) difference was observed (table 16). When the mean values of parameters representing glycemic status were compared statistically among the age group 61-80 yrs in group A & B, Group A & C highly significant (p < .001) difference was observed and when group B & C were compared non significant (p > .05) difference was observed (table 17). These findings in subgroups as per age of study groups A, B and C are in accordance with the findings of comparative analysis of mean values in the major study groups (table 4 & 5).

The mean values of fasting and post prandial glucose when compared statistically among subjects of the three subgroups (on the basis of age) in group A were not significant (p > .05) except when FBS & PPBS were compared in age group 21-40 yrs & 61-80 yrs, and when FBS was compared in Age group 41-60 yrs & 61-80 yrs they were significantly lower in age group 61-80 years (p ≤ .05) (table 12). In group B subgroups (on the basis of age) difference in mean values were not significant (p > .05) (table 13). The mean values of fasting and post prandial glucose
when compared statistically among subjects of the three subgroups (on the basis of Age) in group C, the differences were not significant ($p > .05$) except when FBS & PPBS were compared in age group 41-60 yrs & 61-80 yrs they were significantly lower ($p \leq .05$ & $< 0.001$) in age group 61-80 years (table 14). These findings show a trend of decreasing mean glucose levels with increasing age of patients with statistically significant difference in few of subgroups in control and diabetic groups. This trend may be coincidental as the HbA$_{1c}$ and eAG mean values among same subgroups of age in all three major study groups showed no statistically significant difference (table 13 & 14). The day to day glucose values are more fluctuating compared to HbA$_{1c}$. The second possibility is the difference in dietary intake of calories which is relatively less with increasing age compounded with socioeconomic status that is lowered due to non productivity in older age group.

**RENAL PARAMETERS IN DIABETES:**

Diabetes is now the major cause of end stage kidney failure throughout the world in both developed and emerging nations. Chronic kidney disease usually is silent and undetected until advanced stages with adverse outcomes of kidney failure, cardiovascular disease and premature death. A strong association is well recognized between the presence of diabetes, hypertension, chronic kidney disease and cardiovascular diseases.\textsuperscript{541} Diabetes mellitus is a slow, progressive disease characterized by hyperglycemia. Over time, high blood glucose levels damage millions of nephrons - tiny filtering units within each kidney. As a result, kidneys are unable to maintain the fluid and electrolyte homeostasis. Creatinine is filtered by the glomerulus; therefore, serum creatinine level is used as an indirect measure of glomerular filtration. As glomerular filtration rate (GFR) diminishes, there is a rise in plasma concentrations of serum creatinine and urea. Hyperglycemia is a precondition for developing two major early glomerular lesions, glomerular basement
membrane thickening and mesangial expansion, which are not present at the diagnosis of diabetes but are found 2 to 5 yrs after onset of hyperglycemia. Furthermore, this rise indicates progression of kidney disease and estimation of serum creatinine has greater prognostic ability compared with urea for predicting the adverse outcomes. 

Clinical DN is defined as the development of persistent proteinuria and hypertension, and it is preceded by incipient DN, characterized by persistent microalbuminuria. The degree of microalbuminuria determines the progression of DN. It may reflect the renal manifestation of a global vascular dysfunction. The ‘gold standard’ of microalbuminuria measurement is based on the excretion rate of albumin in a timed urine collection, while for more rapid estimations the urinary albumin concentration in an early morning mid stream specimen of urine may be used. In order to correct for variations in body fluid balance, the latter is normally referenced against the urinary creatinine concentration as the albumin: creatinine ratio (ACR) values. The effect of volume can be avoided entirely by calculation of the ACR in an untimed urine specimen. A value above 30 mg/g (or 0.03ug/mg) suggests that albumin excretion is above 30 mg/day and therefore that microalbuminuria is probably present.

In present study as per table 18 & fig. 28 the mean values of urea & creatinine in diabetics were: group B (T2DM) 24.93 ± 7.96 mg/dl & 0.92 ± 0.24 mg/dl, group C (T2DMN) 45.95 ± 28.10 mg/dl & 1.46 ± 0.92 mg/dl as compared to control (group A) 23.59 ± 3.63 mg/dl & 0.88 ± 0.10 mg/dl. Also the mean values of urine microalbumin in diabetics were group B (T2DM) 11.69 ± 4.09 mg/day, group C (T2DMN) 175.91 ± 234.84 mg/day as compared to control (group A) 9.85 ± 3.49 mg/day. When the mean values of parameters representing renal function were compared statistically in the three groups highly significant (p < .001) difference was observed in all except for creatinine where it was significant (p ≤ .05) &
for Urea where it was not significant (p > .05) when compared in control and diabetics without nephropathy (table 19).

Similar significantly higher values of serum creatinine and urea has been reported in diabetics with and without microvascular complications as compared to control by Mittal A et al\textsuperscript{543}, Gurjeet Singh et al\textsuperscript{544}, Kannel WB et al\textsuperscript{294}, Shaymaa Zahraw Nada et al\textsuperscript{545}, Emre Sinan Gungor et al\textsuperscript{546}, Anupriya Sharma et al\textsuperscript{547}, Whaley-Connell A et al\textsuperscript{541} and others. They found creatinine to be more significant compared to urea levels.

**Elevated serum levels of creatinine and urea are pathogenic of renal insufficiency.** The creatinine level is a more reliable parameter than urea level for identification of renal dysfunction, since the serum level of creatinine rises earlier than that of urea and the formation of creatinine is largely independent of protein metabolism, in contrast to the formation of urea\textsuperscript{548} and because creatinine has lower back diffusion from tubules lumen to peritubular blood\textsuperscript{549}. Raised serum urea and creatinine levels in diabetics clearly indicate that prolonged hyperglycaemia causes irreversible damage to nephrons of kidney. Raised serum creatinine and reduced GFR has become firmly entrenched as fairly reliable indicators of kidney dysfunction. In patients who have normal or elevated renal function but are suspected of losing renal function over time, creatinine-based measures are unreliable for detecting trends\textsuperscript{550}.

Thus microalbuminuria has now been established as more sensitive and specific marker of diabetic nephropathy with increasing values indicator of progression of DN. In present study urinary microalbumin was significantly higher in diabetics compared to control group with highest mean value in diabetics with nephropathy. Various studies by Ramchandran A et al\textsuperscript{436}, DCCT 1993\textsuperscript{113}, UKPDS 33\textsuperscript{114}, R Unnikrishnan et al\textsuperscript{456}, Asselbergs FW et al\textsuperscript{460}, Zelmanovitz T et al\textsuperscript{497} and Nathan DM et al\textsuperscript{513}
have shown strong correlation of microalbuminuria with diabetes and higher values in accordance with progression of nephropathy.

As per Table 20 & fig. 29 the mean values of urea & creatinine in diabetics whose UACR was calculated were: group B (T2DM) 21.58 ± 7.40 mg/dl, & 0.87 ± 0.21 mg/dl, group C (T2DMN) 34.37 ± 15.85 mg/dl & 1.14 ± 0.53 mg/dl as compared to control (group A) 22.69 ± 4.88 mg/dl & 0.83 ± 0.12 mg/dl. Also the mean values of urine microalbumin & urine creatinine in diabetics were-group B (T2DM) 11.64 ± 4.77 mg/day, 65.43 ± 65.33 mg/dl & group C (T2DMN) 181.15 ± 262.22 mg/day, 96.16 ± 96.20 as compared to control (group A) 11.99 ± 4.11 mg/day, 90.04 ± 49.95 mg/dl respectively. The mean value of UACR in diabetics were: group B (T2DM) 29.12 ± 30.67 mg/gm, group C (T2DMN) 294.77 ± 485.83 mg/gm as compared to control (group A) 17.73 ± 12.82 mg/gm. When the mean values of parameters representing renal function in individuals whose UACR was calculated were compared statistically in group A & B the difference was not significant (p > .05) except for UACR where it was significant (p ≤ .05). While comparing group A & C and highly significant (p < .001) difference was observed in all except for urinary Creatinine where it was not significant (p > .05). And when comparing the mean values of all parameters in group B & C highly significant (p < .001) difference was observed in all except for urinary creatininine where it was significant (p ≤ .05) (table 21). UACR was significantly higher in diabetics with and without nephropathy as compared to control group, with highest mean value in DN (group C). Urinary creatinine value alone was relatively less significant as compared to UACR which had strong correlation to diabetes and diabetic nephropathy as that of microalbuminuria. Use of the UACR in an untimed urinary sample is now recommended as the preferred screening strategy for all diabetic patients and can be replaced for microalbuminuria as per the studies done by Mogensen, CE et al.463 Gross JL et al.511 K/DOQI 2007514 and Nathan DM et al.513 While interpreting the values of urinary microalbumin
or UACR the following things must be kept at the back of mind that exercise within 24 hours, infection, fever, congestive heart failure, marked hyperglycemia, marked hypertension, pyuria, and hematuria may elevate urinary albumin excretion rate over baseline values. Albumin excretion will be underestimated in a muscular man with a high rate of creatinine excretion and overestimated in a cachectic patient in whom muscle mass and creatinine excretion may be markedly reduced. To overcome these fallacies, confirmation of microalbuminuria or raised UACR values on two successive occasions measured after specific period have been advocated for diagnosing diabetic nephropathy.

The mean values of urea, & creatinine in diabetics with microalbuminuria (group C I) were 45.16 ± 28.63 mg/dl & 1.40 ± 0.83 mg/dl & in diabetics with macroalbuminuria (group C II) were 49.31 ± 25.94 mg/dl & 1.68 ± 1.24 mg/dl. Whereas the mean values of microalbumin in diabetics with microalbuminuria (group C I) was 73.02 ± 55.42 mg/day & in diabetics with macroalbuminuria (group C II) was 611.49 ± 201.83 mg/day (table 22 & fig. 30). The above data shows that the mean values of urea & creatinine in diabetics with macroalbuminuria (Group C II) were high as compared to diabetics with microalbuminuria (Group C I) but the difference was not significant statistically (p > .05). Whereas the difference in mean values of urine microalbumin was highly significant statistically (p < .001). Similar findings were seen in groups A, B and C patients in whom urinary creatinine and UACR values were measured. The differences in UACR mean values were highly significant statistically (p < .001) (table 23 & fig. 31). To summarize: the mean values of urinary microalbumin and UACR were significantly different among diabetics with incipient nephropathy (microalbuminuria) and diabetics with overt nephropathy (macroalbuminuria), while urea and creatinine mean values had no statistically significant difference to differentiate the two groups of diabetic nephropathy. Hence measurement of Urine microalbumin and UACR are better predictors of renal damage at an
early stage. The increasing values of albuminuria with progression of DN has been reported by Asselbergs FW et al\textsuperscript{460}, Ritz E et al\textsuperscript{506}, Nathan DM et al\textsuperscript{513}, Zelmanovitz T et al\textsuperscript{497}, ADA 2003\textsuperscript{495}, Mogensen CE et al\textsuperscript{502} and Bahman P T et al.\textsuperscript{538} Gilbert RE et al\textsuperscript{483} has shown positive correlation of poor glycemic control with increasing microalbuminuria and overt diabetic nephropathy.

On comparing the mean values of renal parameters statistically among the male and female subjects in group A & C, group B & C respectively, highly significant (p < .001) difference was observed. When the mean values were compared among the male subjects of group A & B the difference was not significant (p > .05) \textit{(table 26)} and when the mean values were compared among the female subjects of group A & B the difference was not significant (p > .05) for creatinine, significant (p \leq .05) for urea and highly significant (p < .001) for urinary microalbumin \textit{(table 27)}. The mean values of renal parameters when compared statistically among both the male & female subjects in group B (T2DM) and in group C (T2DMN), the differences were not significant statistically (p > .05), except for Creatinine in group C which was highly significant (p \leq .001). In group A when the mean values of serum urea, creatinine and urine microalbuminuria were compared statistically among the male & female subjects the difference was highly significant (p < .001) \textit{(table 24, 25 & fig. 32)}. Thus in control group the males had higher mean serum creatinine and urea values compared to females, which is explainable due to higher muscle mass and effectively more protein turnover in males compared to females. Also the other confounding factors related to microalbumin like history of smoking\textsuperscript{430}, hypertension \textsuperscript{431} and obesity\textsuperscript{432} may explain higher levels of urinary microalbumin in males \textbf{compared to females} as also supported by studies of Parving H et al\textsuperscript{443}, Niskanen LK et al\textsuperscript{551}, Forsblom CM et al\textsuperscript{552} and Massoud Amini et al\textsuperscript{553}. 

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The gender based differences in renal parameters among the 3 study groups i.e control (group C), diabetics without nephropathy (group A) and diabetics with nephropathy (group B) were similar to those found among the main study groups.

As per table 28 & fig. 33 the mean values of urea in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 25.55 ± 11.58 mg/dl, 24.40 ± 7.21 mg/dl, 26.32 ± 8.37 mg/dl & group C (T2DMN) were 41.47 ± 27.65 mg/dl, 45.60 ± 31.07 mg/dl, 48.05 ± 17.65 mg/dl as compared to group A (control) 23.16 ± 2.97 mg/dl, 23.42 ± 3.43 mg/dl, 24.16 ± 4.29 mg/dl respectively. The mean values of creatinine in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 0.93 ± 0.28 mg/dl, 0.90 ± 0.16 mg/dl, 1.01 ± 0.38 mg/dl & group C (T2DMN) were 1.09 ± 0.37 mg/dl, 1.46 ± 1.00 mg/dl, 1.52 ± 0.76 mg/dl as compared to group A (control) 0.85 ± 0.12 mg/dl, 0.87 ± 0.09 mg/dl, 0.91 ± 0.12 mg/dl respectively. The mean values of urine microalbumin in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 10.48 ± 3.17 mg/day, 11.95 ± 4.32 mg/day, 11.47 ± 3.67 mg/day & group C (T2DMN) were 75.82 ± 82.05 mg/day, 193.61 ± 256.70 mg/day, 148.38 ± 181.10 mg/day as compared to control group A (control) 7.61 ± 1.02 mg/day, 10.07 ± 3.44 mg/day, 10.30 ± 3.95 mg/day respectively.

The mean values of parameters representing renal function when compared statistically among subjects of the three subgroups (on the basis of age) in group A (controls) and group B (T2DM) respectively were not significant (p >.05) except when in group A urine microalbumin was compared in age group 21-40 yrs & 41-60 yrs, and when it was compared in age group 21-40 yrs & 61-80 yrs the difference was highly significant statistically (p < .001) (table 29 & 30). The mean values of renal parameters when compared statistically among subjects of the three subgroups (on the basis of Age) in group C, the differences were not significant (p >.05). Except
when creatinine and urine microalbumin were compared in the age group 21-40 yrs & 41-60 yrs the difference was significant (p ≤ .05) and when creatinine was compared in age group 21-40 yrs & 61-80 yrs it was also significant (p ≤ .05) statistically (table 31). **Thus in diabetics with nephropathy (group C) the serum creatinine showed increasing values with increasing age and duration of DM which goes well with the decreasing GFR leading to increased serum creatinine with increasing stage of DN.** The increasing mean values of urinary microalbumin in diabetics without nephropathy with increasing age of patients correlates with the findings of various studies by Shehnaz A Sheikh et al\textsuperscript{11}, Ramchandran A et al\textsuperscript{436}, DCCT 1993\textsuperscript{113}, UKPDS 33\textsuperscript{114} and R Unnikrishnan et al\textsuperscript{456} that relate **increased values of urinary microalbumin with patient age, duration of diabetes** and other factors like hypertension, smoking and obesity.

When the mean values of parameters representing renal function were compared statistically among the age group 21-40 yrs in Group A & B, Group A & C and Group B & C the difference was not significant (p > .05) for urea and creatinine whereas it was significant (p ≤ .05) for microalbumin (table 32). When the mean values of renal parameters were compared statistically among the age group 41-60 yrs and 61-80 yrs in Group A & C and Group B & C highly significant (p < .001) difference was observed for all the parameters. When the mean values of renal function tests were compared in Group A & B statistically significant (p ≤ .05) difference was observed for urine microalbumin in age group 41-60 years whereas for urea & creatinine it was not significant (p > .05) (table 33 & 34).

The findings in study groups A, B and C as per age subgroups were similar to those found in the major study groups covering patients and control subjects of all age groups. As none of the diabetic patients in age group 21-40 years had overt nephropathy (microalbuminuria ≥ 300mg/day) as
compared to age group 41-60 yrs and 61-80 yrs, they were in initial stages of diabetic nephropathy which was demarcated by significant difference in urinary microalbumin mean values but not depicted by statistically significant difference in urea and creatinine mean values that typically rise with advanced stages of DN. These findings further strengthen the predictive value of microalbumin in incipient and overt DN and the findings of other workers (Tejal J Wagle, Mittal A et al, Muhammad Yakoob Ahmedani et al, and Whaley-Connell A et al) that microalbuminuria is related to age of patient and duration of diabetes. Though urinary microalbumin is a reliable parameter to detect diabetic nephropathy, we cannot rely on it alone as Studies by Kramer HJ et al and MacIsaac RJ et al have shown that abnormal GFR values and urinary albuminuria may not be simultaneously present in up to 40% of patients with DM and chronic renal disease. There are several explanations to possibly account for this. Firstly, as per study by Steele DJ et al and Lane PH et al pathologically proven diabetic nephropathy can exist in the absence of urinary albuminuria, though this finding is unusual. Secondly, as per Ruggenenti P et al the cause of chronic renal disease in patients with DM is heterogeneous and renal dysfunction is often associated with other concurrent diseases other than diabetic nephropathy such as hypertension or reno-vascular disease. The ADA recommends the routine screening for diabetic nephropathy and chronic renal disease in patients with DM using both urine albumin excretion and serum creatinine measurements.

LIPIDS IN DIABETES

Epidemiologic studies have demonstrated that diabetes mellitus is an independent risk factor for cardiovascular disease and that it amplifies the effects of other common risk factors, such as smoking, hyper tension and hypercholesterolemia. The precise pathogenesis of diabetic dyslipidemia is
not known; nevertheless, a large body of evidence suggests that insulin resistance has a central role in the development of this condition.

The most common pattern of dyslipidemia in patients with type 2 diabetes is elevated triglyceride levels and decreased HDL cholesterol levels. The mean concentration of LDL cholesterol in those with type 2 diabetes is not significantly different from that in those individuals who do not have diabetes. However, qualitative changes in LDL cholesterol may be present. In particular, patients with diabetes tend to have a higher proportion of smaller and denser LDL particles, which are more susceptible to oxidation and may thereby increase the risk of cardiovascular events. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance. Available prospective cohort studies suggest that lipid abnormalities are associated with increased risk of cardiovascular events in patients both with and without diabetes. Various studies have demonstrated that LDL, HDL, and triglycerides are independent predictors of CVD.

In the present study (table 35 & figure 34) the mean values of cholesterol & triglycerides in diabetics were group B (T2DM) 182.29 ± 44.78 mg/dl & 154.91 ± 82.14 mg/dl, group C (T2DMN) 187.40 ± 52.93 mg/dl & 177.77 ± 112.30 mg/dl as compared to control (group A) 185.22 ± 35.31 mg/dl & 128.30 ± 60.14 mg/dl. The mean values of HDL & LDL in diabetics were group B (T2DM) 40.91 ± 8.17 mg/dl & 113.40 ± 33.26 mg/dl, group C (T2DMN) 39.96 ± 7.75 mg/dl & 113.84 ± 40.91 mg/dl as compared to control (Group A) 44.97 ± 6.62 mg/dl & 116.32 ± 27.44 mg/dl. The mean values of total cholesterol and HDL (Chol:HDL) ratio & LDL:HDL ratio in diabetics were group B (T2DM) 4.54 ± 1.11 and 2.81 ± 0.96, group C (T2DMN) 4.80 ± 1.48 and 2.60 ± 1.04 as compared to control (group A) 4.16 ± 0.88 and 2.61 ± 0.77. The mean values of VLDL in diabetics were group B (T2DM) 30.98 ± 16.42 mg/dl, group C (T2DMN) 35.56 ± 22.47 mg/dl as compared to control (group A) 25.66 ± 12.02 mg/dl. When the
mean values of parameters of lipid profile were compared statistically among group A & B and group A & C highly significant (p < .001) difference was observed for all except LDL: HDL ratio where it was significant (p ≤ .05) and cholesterol & LDL where it was not significant (p > .05). When the mean values of parameters of lipid profile were compared statistically in group B & C non significant (p > .05) difference was observed for all except triglycerides & VLDL where it was significantly higher in group C (p ≤ .05) (table 36).

Similar findings of hypertriglyceridemia, low HDL and high VLDL mean values in diabetics compared to non diabetics have been reported by Framingham Heart Study294, UKPDS study295, Pyorala K et al117, Gaede P et al118 and Christa Meisinger et al.119 Diabetics with nephropathy showed significant higher mean values of triglycerides and VLDL compared to diabetics without nephropathy which is consistent with the findings of Syed Muhammad Shahid et al123, Haseeb Ahmad Khan124 and M. Rema et al.125 The precise pathogenesis of diabetic dyslipidemia is unknown. Various studies suggest that insulin resistance has a central role in the development of this condition.290-293 The main cause of the three cardinal features of diabetic dyslipidemia is the increased free fatty-acid release from insulin-resistant fat cells.290-293

The increased flux of free fatty acids into the liver in the presence of adequate glycogen stores promotes triglyceride production, which in turn stimulates the secretion of apolipoprotein B (ApoB) and VLDL. The impaired ability of insulin to inhibit free fatty-acid release leads to enhanced hepatic VLDL cholesterol production,296 which correlates with the degree of hepatic fat accumulation.297 Hyperinsulinemia is also associated with low HDL cholesterol levels.298,299 The increased number of VLDL cholesterol particles and increased plasma triglyceride levels decrease the level of HDL cholesterol and increase the concentration of small dense LDL cholesterol particles via several processes: VLDL-
transported triglyceride is exchanged for HDL-transported cholesteryl ester through the action of the cholesteryl ester transfer protein (CETP), which results in increased amounts of both atherogenic cholesterol-rich VLDL remnant particles and triglyceride-rich, cholesterol-depleted HDL particles.

As per table 37 & figure 35 the mean values of cholesterol and triglycerides in diabetics with microalbuminuria were (group C I) 181.46 ± 46.33 mg/dl & 170.17 ± 115.98 mg/dl & in diabetics with macroalbuminuria were (group C II) 212.57 ± 70.23 mg/dl & 209.93 ± 89.83 mg/dl. The mean values of HDL and LDL in diabetics with microalbuminuria were (group C I) 39.69 ± 7.28 mg/dl & 110.07 ± 38.21 mg/dl & in diabetics with macroalbuminuria (group C II) 41.13 ± 9.54 mg/dl & 129.80 ± 48.33 mg/dl. Also the mean ratios of total cholesterol: HDL cholesterol and LDL: HDL ratio where it was not significant (p > .05) statistically. Similar higher mean values of triglycerides, cholesterol and VLDL in diabetics with macroalbuminuria as compared to microalbuminuria has been reported by Pyorala K et al\textsuperscript{117}, Gaede P et al\textsuperscript{118}, Christa Meisinger et al\textsuperscript{119} and V Mohan et al\textsuperscript{121}. Diabetic nephropathy (DN) is associated with an altered lipid profile characterized by elevated triglyceride rich lipoproteins, in particular VLDL, but also LDL and thus, plasma triglycerides are
The levels of HDL are low as a secondary phenomenon. There still seems to be uncertainty on the underlying mechanisms, but changes in lipoprotein lipase (LPL) and hepatic lipase (HL) have been suggested. An increased HL-activity and a reduced post heparin plasma LPL/HL ratio have been reported. These multiple lipoprotein alterations become more accentuated with declining renal function and increasing urinary albumin excretion.

The mean values of cholesterol in male & female subjects (table 38 & figure 36) of group B (T2DM) were 177.55 ± 45.26 mg/dl, 187.40 ± 43.97 mg/dl & group C (T2DMN) were 182.26 ± 46.15 mg/dl, 195.72 ± 61.86 mg/dl as compared to control (group A) 180.20 ± 36.83 mg/dl, 190.19 ± 33.22 mg/dl respectively. The mean triglyceride values in male & female subjects of group B (T2DM) were 159.96 ± 91.50 mg/dl, 149.47 ± 70.89 mg/dl & group C (T2DMN) 172.68 ± 111.07 mg/dl, 186.0 ± 114.71 mg/dl as compared to control (group A) 139.67 ± 76.50 mg/dl, 117.07 ± 34.56 mg/dl respectively. Also the mean values of HDL in male & female subjects of group B (T2DM) were 38.62 ± 8.25 mg/dl, 43.38 ± 7.37 mg/dl & group C (T2DMN) 38.84 ± 7.04 mg/dl, 41.78 ± 8.52 mg/dl as compared to control (group A) 43.20 ± 6.44 mg/dl, 46.72 ± 6.36 mg/dl respectively. The mean LDL values in male & female subjects of group B (T2DM) were 111.71 ± 32.24 mg/dl, 115.22 ± 34.43 mg/dl & group C (T2DMN) were 113.24 ± 38.16 mg/dl, 114.82 ± 42.33 mg/dl as compared to control (group A) 112.68 ± 26.87 mg/dl, 119.90 ± 27.68 mg/dl respectively. The mean values of total cholesterol: HDL cholesterol ratio in male & female subjects of group B (T2DM) were 4.71 ± 1.26, 4.35 ± 0.90 & group C (T2DMN) 4.78 ± 1.30, 4.84 ± 1.74 as compared to control (group A) 4.20 ± 0.82, 4.12 ± 0.95 respectively. The mean LDL: HDL ratio in male & female subjects of group B (T2DM) were 2.95 ± 1.12, 2.66 ± 0.72 & group C (T2DMN) were 2.94 ± 0.97, 2.82 ± 1.14 as compared to control group A 2.58 ± 0.75, 2.64 ± 0.79 respectively. The mean values of VLDL in male & female subjects of group B (T2DM) were 31.99 ± 18.30
mg/dl, 29.89 ± 14.17 mg/dl & group C (T2DMN) were 34.54 ± 22.21 mg/dl, 37.22 ± 22.98 mg/dl as compared to control group A 27.93 ± 15.30 mg/dl, 23.41 ± 6.91 mg/dl respectively (table 38).

When the mean values of parameters of lipid profile were compared statistically among the male & female subjects in group A, B & C the difference observed was not significant (p > .05) except for HDL which was higher in females, with highly significant difference (p < .001) in group A, B and significant difference (p ≤ .05) in group C. Also in group A triglyceride and VLDL were significantly higher in males (p ≤ .05) and in group B total cholesterol: HDL cholesterol ratio was significantly higher in males (p ≤ .05) (table 39).

When the mean values of parameters of lipid profile were compared statistically among the male subjects in group A & B, HDL was decreased in diabetics and the difference was highly significant (p < .001), total cholesterol: HDL cholesterol and LDL:HDL ratio were significantly higher in diabetics (p ≤ .05) and others were not significant (p > .05). In group A & C highly significant (p < .001) difference was observed for HDL & total cholesterol: HDL cholesterol ratio and significant (p ≤ .05) difference was observed for triglycerides, LDL: HDL ratio & VLDL. In group B & C the difference for all the parameters was not significant statistically (p > .05) (table 40).

When the mean values of parameters of lipid profile were compared statistically among the female subjects in group A & B, triglycerides & VLDL were increased in diabetic females with highly significant difference compared to control group females and HDL was significantly lower in diabetic females (p ≤ .05). In group A & C highly significant (p < .001) difference was observed for triglycerides, HDL and VLDL whereas difference in mean values of total cholesterol: HDL cholesterol and LDL: HDL ratio was significant (p ≤ .05) and others were not significant (p > .05). In group B & C the mean values of triglycerides & VLDL were
significantly higher in diabetic females with nephropathy as compared to diabetics without nephropathy (p ≤ .05) and the difference in mean values of other parameters were not significant (p > .05) (table 41).

Thus the male and female subgroups in all 3 study groups showed significantly higher triglycerides and VLDL mean values and lower HDL mean values in diabetics with and without nephropathy as compared to non diabetic controls. Total cholesterol levels of those with diabetes mellitus and control individuals did not differ. Similar findings in male and female type 2 DM patients have been reported in Framingham Heart Study\textsuperscript{294} and UKPDS study.\textsuperscript{295} Arshag D Mooradian\textsuperscript{562} and H.A. Elnasri et al.\textsuperscript{563}

As per table 42 & figure 37 the mean values of cholesterol in the age group 21-40 yrs, 41-60 yrs and 61-80 yrs of the subjects of group B (T2DM) were 192.13 ± 41.55 mg/dl, 181.76 ± 44.27 mg/dl & 179.49 ± 47.90 mg/dl & group C (T2DMN) were 204.89 ± 66.60 mg/dl, 187.65 ± 56.45 mg/dl & 182.55 ± 36.93 mg/dl as compared to control (group A) 196.44 ± 44.05 mg/dl, 189.78 ± 34.32 mg/dl & 169.98 ± 29.21 mg/dl respectively. The mean values of triglycerides in the age group 21-40 yrs, 41-60 yrs and 61-80 yrs of the subjects of group B (T2DM) were 140.31 ± 162.62 mg/dl, 162.41 ± 90.34 mg/dl & 137.80 ± 56.82 mg/dl & group C (T2DMN) were 181.11 ± 127.92 mg/dl, 187.88 ± 117.51 mg/dl & 147.71 ± 87.82 mg/dl as compared to control (group A) 160.78 ± 73.01 mg/dl, 129.05 ± 65.74 mg/dl & 113.27 ± 27.59 mg/dl respectively. The mean values of HDL in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 43.88 ± 6.37 mg/dl, 40.55 ± 8.00 mg/dl & 40.71 ± 9.31 mg/dl & group C (T2DMN) were 43.11 ± 9.99 mg/dl, 39.58 ± 8.42 mg/dl & 40.32 ± 4.46 mg/dl as compared to control (group A) 47.83 ± 6.26 mg/dl, 45.84 ± 6.39 mg/dl & 41.75 ± 6.24 mg/dl respectively. The mean values of LDL in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 122.00 ± 31.06 mg/dl, 112.38 ± 32.20 mg/dl & 112.71 ± 37.66 mg/dl & group C (T2DMN) were 115.78 ± 43.03
mg/dl, 112.83 ± 40.84 mg/dl & 116.32 ± 42.65 mg/dl as compared to control (group A) 118.50 ± 32.00 mg/dl, 120.20 ± 26.74 mg/dl & 106.32 ± 25.10 mg/dl respectively. The mean values of total cholesterol: HDL cholesterol ratio in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 4.41 ± 0.87, 4.59 ± 1.23 & 4.45 ± 0.78 & group C (T2DMN) were 5.18 ± 2.77, 4.86 ± 1.50 & 4.55 ± 0.92 as compared to control (group A) 4.01 ± 0.86, 4.17 ± 0.87 & 4.23 ± 0.94 mg/dl respectively. The mean values of LDL: HDL ratio in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 2.82 ± 0.77, 2.83 ± 1.05 & 2.77 ± 0.68 & group C (T2DMN) were 2.85 ± 1.32, 2.91 ± 1.05 & 2.87 ± 0.96 as compared to control (group A) 2.55 ± 0.52, 2.62 ± 0.76 & 2.63 ± 0.89 respectively. The mean values of VLDL in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 28.06 ± 12.52 mg/dl, 32.48 ± 18.06 mg/dl & 27.56 ± 11.36 mg/dl & group C (T2DMN) were 36.33 ± 25.86 mg/dl, 37.57 ± 23.50 mg/dl & 29.54 ± 17.56 mg/dl as compared to control (group A) 32.15 ± 14.60 mg/dl, 25.81 ± 13.14 mg/dl & 22.65 ± 5.51 mg/dl respectively.

The mean values of parameters of lipid profile when compared statistically among subjects of the three subgroups (on the basis of age) in group A, the differences were not significant (p > .05) in the age group 21-40 yrs & 41-60 yrs. Whereas among age groups 21-40 yrs & 61-80 yrs cholesterol, triglycerides, HDL & VLDL were significantly higher in age group 21-40 years (p ≤ .05) and the difference in other parameters were not significant (p > .05). Among the age groups 41-60 yrs & 61–80 yrs, higher mean values were seen in age group 41-60 yrs that were highly significant for cholesterol & HDL (p < .001), and significant for triglycerides, LDL and VLDL (p ≤ .05). While the differences in other parameters were not significant (p > .05) statistically (table 43). The mean values of parameters of lipid profile when compared statistically among subjects of the three subgroups (on the basis of age) in group B, the differences were not significant (p > .05) statistically in any of the subgroups (table 44). The
mean values of parameters of lipid profile when compared statistically among subjects of the three subgroups (on the basis of age) in group C showed statistically non-significant difference (p > .05) in the age group 21-40 yrs & 41-60 yrs and 21-40 yrs & 61-80 yrs. Whereas among age groups 41-60 yrs & 61-80 yrs triglycerides, & VLDL were significantly higher (p ≤ .05) in age group 41-60 yrs. The difference in mean values of other parameters were not significant (p > .05) (table 45).

When the mean values of parameters of lipid profile were compared statistically among the age group 21-40 yrs in group A & B, group A & C and group B & C the difference observed was not significant (p > .05) statistically (Table 46). On comparing the mean values of parameters of lipid profile statistically among the age group 41-60 yrs in group A & B, and group A & C, lower value was observed for HDL in diabetics with highly significant difference statistically (p < .001) and significantly (p ≤ .05) higher values in diabetics (group B & C) was observed for triglycerides, total cholesterol: HDL cholesterol ratio and VLDL as compared to control group A, whereas not significant difference was observed for cholesterol, LDL: HDL ratio and LDL except for group C where LDL:HDL ratio was significantly higher compared to control group. In group B & C all the parameters of lipid profile showed no significant difference in mean values (p > .05) (table 47). When the mean values of parameters of lipid profile were compared statistically among the age group 61-80 yrs in group A & B and group A & C significantly higher (p ≤ .05) values were observed for triglycerides & VLDL in diabetics (group B & C), whereas other parameters showed no significant difference (p > .05).

When the mean values of parameters of lipid profile in group B & C were compared statistically not significant (p > .05) difference was observed (table 48). **Thus on comparing the lipid profile parameters among subgroups based on age in the same study groups, only the control group showed significantly higher values in age group 21-40 yrs and 41-60 yrs as compared to 61-80 yrs age group. This may be explained**
by higher dietary intake of fat in younger and middle age groups as compared to older age group people. No such statistical difference was seen among different age groups in diabetics with and without nephropathy (group B & C), supporting the hypothesis of dyslipidemia present from onset of type 2 DM and persisting throughout the disease course.

Anthonia O Ogbera et al found that clinical parameters like age, sex, duration of DM, and glycaemic control were not possible determinants of the presence of an abnormal lipid profile. Elevated triglyceride was more significantly elevated in the middle and elderly age group than in the younger age group in study by Anthonia O Ogbera et al, H.A Elnasri et al and Mats Eriksson et al. Mats Eriksson et al found that LDL:HDL ratios decreased with age in both sexes.

In study by Hardev Singh Sandhu et al, in males type 2 DM for serum cholesterol, triglycerides & HDL, the maximum mean value was noted in middle age group and the minimum in younger age group for cholesterol and triglycerides and older age group for HDL. For LDL, almost equal distribution was recorded in all the age groups. For serum cholesterol, in females the maximum mean value was found in younger age group and the minimum in older age group. For HDL, the maximum mean value was recorded in middle age group and the minimum in older age group. For LDL, the maximum mean value was found in middle age group and the minimum in younger age group. For triglyceride, the maximum mean value was noted in middle age group and the minimum in older age group.

In study by H A Elnasri et al the levels of total cholesterol showed a progressive increase with age up to the age of 60 years. The differences in the values of total cholesterol, triglycerides and HDL between the youngest and oldest age groups were statistically significant. Their study indicates a positive association between age and lipid abnormalities, with higher levels among older than younger ages. This fact, to some extent, may hold true
for non diabetic subjects. Some studies indicate that the level of triglycerides decreases after the sixth decade, stating reasons such as lower food intake and absorption and increased rate of catabolism.

Comparison of lipid profile parameters among same age group patients in group A, B & C showed non significant difference in age group 21-40 yrs, while the other two age groups (41-60yrs & 61-80 yrs) showed similar trend as that was seen in the comparison of major study groups A, B & C i.e dyslipidemia in form of decreased HDL and increased triglycerides, VLDL & cholesterol:HDL ratio in diabetics as compared to control group. No significant difference was seen among diabetics with and without nephropathy in all age groups.

**ACUTE PHASE PROTEINS**

Two decades ago, Crook et al showed that, in comparison with non diabetic subjects, circulating concentrations of commonly recognized acute-phase reactants were increased in type II but not type I diabetic patients who were matched for age, sex, glycemic control, and the absence of tissue complications. These acute-phase reactants included CRP, serum amyloid A, α 1-acid glycoprotein, and sialic acid. Inflammation plays a major role in the pathogenesis of type 2 diabetes mellitus and its complications. Hence inflammatory markers or acute phase markers have gained the importance as indicators and predictors of diabetic process. In the past decade, it has become widely accepted that inflammation plays a key role in the pathogenesis of CVD. CRP, an APP and marker of chronic, low-grade inflammation, is a reliable predictor of CVD. Data from prospective, epidemiologic studies revealed a significant association between CRP and future CHD risk in apparently healthy subjects. Similarly, high plasma CRP has been shown to be an independent risk factor for CHD deaths in type II DM patients.
Serum sialic acid is considered as a marker of innate immunity and activated innate immunity is a risk factor for CVD in type II DM.\textsuperscript{191} It is known that ceruloplasmin has antioxidant properties because of its ferroxidase activity. Alternatively, ceruloplasmin is thought to be a scavenger of ROS\textsuperscript{272} and plays an important role in nitrosothiol formation, which may contribute to its potent antioxidant activities.\textsuperscript{273} Recently unexpected, prooxidant effects of plasma ceruloplasmin were demonstrated by some authors. An increase in serum ceruloplasmin in type II DM could generate excess oxidized LDL, which causes atherosclerosis.\textsuperscript{274} These observations support the hypothesis that copper bound at specific sites on protein surfaces can cause oxidative damage to macromolecules in their environment.\textsuperscript{275}

In the present study table 49 & figure 38 show the mean values of TSA in diabetics were group B (T2DM) 78.83 \(\pm\) 6.95 mg/dl, group C (T2DMN) 82.67 \(\pm\) 6.63 mg/dl as compared to control (group A) 60.68 \(\pm\) 4.92 mg/dl. Also the mean values of ceruloplasmin & hsCRP in diabetics group B (T2DM) were 35.65 \(\pm\) 3.83 mg/dl & 2.07 \(\pm\) 1.32 mg/L, group C (T2DMN) were 44.37 \(\pm\) 4.53 mg/dl & 3.20 \(\pm\) 1.44 mg/L as compared to control (group A) 26.64 \(\pm\) 2.43 mg/dl & 1.16 \(\pm\) 0.50 mg/L. When the mean values of parameters representing Acute Phase Proteins were compared statistically in the three groups' highly significant (p < .001) difference was observed for all with higher mean values in diabetics (group B & C) as compared to control group and highest values in diabetic nephropathy patients (group C). Similar increased values of APP have been documented in type 2 DM by Crook et al\textsuperscript{203} (CRP & TSA), Soinio, M et al\textsuperscript{215} (hsCRP), A Melidonis et al\textsuperscript{236} (TSA), A. Merat et al\textsuperscript{156}, J Chen et al\textsuperscript{24}, B Shivananda Nayak et al\textsuperscript{569} (TSA), Martha RM et al\textsuperscript{217} (CRP), Walter RM et al\textsuperscript{277}, Cunningham J et al\textsuperscript{278}, McMillan DE et al\textsuperscript{280} (ceruloplasmin). It is perceived that chronic low-grade inflammation as evidenced by elevated hs-CRP might potentially be a cause underlying the etiology and manifestation of type II DM, although the exact mechanisms are
still not well understood. Martha RM et al in their study found that hyperglycemia is an associated factor to the increase of serum CRP levels; in uncontrolled type 2 diabetes subjects. Luciana ML et al and Safiullah Amanullah et al in their study found that hypertensive patients with type 2 DM had higher levels of hs-CRP, a circulating inflammatory marker, than normal subjects. This finding suggests that patients with two associated diseases have a more active inflammatory state. Several studies demonstrate that hs-CRP remained a significant predictor of diabetes risk even after adjusting with BMI, family history of DM, smoking and other factors. Chiriboga DE et al, Jager A et al and Pfutzner A et al in their studies showed that in people with diabetes, CRP levels in highest tertile (> 0.28 mg/dl) were associated with a 2 fold increase in cardiovascular mortality after adjusting for age, sex and glucose tolerance tests. Masuda et al have shown that serum TSA reflects the status of blood glucose control and the progression of ischemic disease of the lower extremities in type 2 DM. Zahedi et al have found that TSA increased post prandially giving further insight as to why it is considered to be a cardiovascular risk factor. Serum TSA is a newly established potential risk factor for the development of macrovascular and microvascular complications of diabetes. Ehrenwald E et al showed that an increase in serum ceruloplasmin in type II DM could generate excess oxidized LDL, which causes atherosclerosis. Starkebaum G et al hypothesised that ceruloplasmin could also cause vascular injury by generating free radicals, such as hydrogen peroxide($H_2O_2$), in the course of oxidization of serum homocysteine. Additional cross-sectional studies in newly diagnosed or established type II diabetes patients have confirmed that inflammatory and acute-phase markers such as CRP, IL-6, TNF-$\alpha$, nitric oxide, ceruloplasmin, ferritin, sialic acid and others are elevated in these subjects compared with nondiabetic control subjects.

Table 51 & figure 39 show the mean values of TSA, ceruloplasmin & hsCRP in diabetics with microalbuminuria (group C I) as 80.30 ± 4.84
mg/dl, 43 ± 3.89 mg/dl & 2.72 ± 1.14 mg/L & in diabetics with macroalbuminuria (group C II) as 92.70 ± 2.44 mg/dl, 50.19 ± 1.20 mg/dl & 5.24 ± 0.50 mg/L respectively. The above data shows that the mean values of parameters representing APP in diabetics with macroalbuminuria (group C II) were high as compared to diabetics with microalbuminuria (group C I) & the difference was highly significant (p < .001) statistically. These observations suggest that low-grade inflammation, reflected by high APP levels, may play a role in the induction of microalbuminuria, which can be considered as a risk factor of cardiovascular diseases. Possible explanations for our finding of an association of markers of inflammation with proteinuria in diabetic nephropathy are threefold. First, elevated levels of inflammatory markers may be the result of pre-existing atherosclerosis in patients with microalbuminuria. In nondiabetic individuals as well as patients with type 2 DM, microalbuminuria is associated with increased cardiovascular morbidity and mortality, suggesting that in individuals with albuminuria, atherosclerotic disease prevails. Second, elevations of acute-phase reactants and/or inflammatory cytokines may directly alter glomerular function and thus be causally involved in the development of albuminuria. Third, there is a potential link between inflammatory cytokines and glomerular function. Pfutzner A et al showed that hs-CRP levels increase with the stage of beta-cell dysfunction and insulin resistance. Martha RM et al showed that hyperglycemia is an associated factor to the increase of serum CRP levels, in uncontrolled type 2 diabetic subjects. Study by Nikhil Choudhary et al and Mohammad Javad Mojahedi et al show similar higher values of hsCRP in diabetics with nephropathy in proportion to proteinuria. In study by B Shivananda Nayak et al there was an insignificant increase in the CRP levels amongst the diabetic groups. This could be explained by the inherent inflammatory state in diabetics with and without complications. While TSA showed significant higher values in diabetic nephropathy patients
with increasing trend corresponding to albuminuria, similar findings have 
been stated by J Chen et al\textsuperscript{24}, Krishnamurthy U et al\textsuperscript{571}, Syed Muhammad 
Shahid et al\textsuperscript{252} and Shivananda Nayak B et al.\textsuperscript{572} Syed Muhammad Shahid 
et al\textsuperscript{252} in their study found increased Serum TSA as a potential risk factor 
for the development of macro and microvascular complications of diabetes. 
It is well established that vascular endothelium carries a high concentraton of SA, and vascular damage could account for its 
shedding into the circulation. As the majority of serum SA is a 
component of glycoproteins, such as acute phase proteins, it is 
important that several serum acute phase proteins are elevated in 
diabetes, particularly in patients with microvascular complications.\textsuperscript{236} 
These observations indicate that SA plays an important role in the 
pathophysiology of endothelial dysfunction and vascular disease in 
DM, and in part explain the increased levels of SA in diabetic 
nephropathy. An increase in serum ceruloplasmin levels has also been 
reported in type 2 DM by Walter RM et al\textsuperscript{277}, Cunningham J et al\textsuperscript{278} and 
McMillan DE et al.\textsuperscript{280} They reported that blood HbA\textsubscript{1c} levels, duration of 
type 2 DM, patient age, and the presence or absence of diabetes 
complications are not major factors influencing ceruloplasmin increase. 

As per table 52 & figure 40 the mean values of TSA in male & female 
subjects of group B (T2DM) were 74.22 ± 7.16 mg/dl, 73.41 ± 6.74 mg/dl & group C (T2DMN) were 82.61 ± 6.52 mg/dl, 82.76 ± 6.85 mg/dl as 
compared to control (group A) 61.58 ± 4.53 mg/dl, 59.79 ± 5.16 mg/dl respectively. The mean ceruloplasmin values in male & female subjects of 
group B (T2DM) were 35.71 ± 4.03 mg/dl, 35.59 ± 3.63 mg/dl & group C (T2DMN) were 44.55 ± 4.47 mg/dl, 44.08 ± 4.64 mg/dl as compared to 
control (group A) 27.41 ± 2.27 mg/dl, 25.88 ± 2.35 mg/dl respectively. Whereas hsCRP in male & female subjects of group B (T2DM) were 2.07 
± 0.96 mg/L, 2.08 ± 1.64 mg/L & group C (T2DMN) were 3.27 ± 1.45 
mg/L, 3.09 ± 1.44 mg/L as compared to control (group A) 1.33 ± 0.50 
mg/L, 1.00 ± 0.43 mg/L respectively.
The mean values of parameters representing APP when compared statistically among male & female subjects in diabetics (group B and group C) were not significant (p > .05) whereas in control (group A) were significantly higher in males [ceruloplasmin, hsCRP (p < .001) and TSA (p ≤ .05)]. Similar findings for hsCRP have been stated by Fatma G. Huffman et al\textsuperscript{212} and Mukta N Chowta et al\textsuperscript{573} and for ceruloplasmin by V O Mabayoje et al.\textsuperscript{574} While Anubha Mahajan et al\textsuperscript{20} and Abdella N et al\textsuperscript{575} in their studies found higher mean value of hsCRP and TSA respectively in female control and diabetics compared to male control and diabetics. When the mean values of parameters representing APP were compared statistically among the male subjects and female subjects in Group A & B, Group A & C and Group B & C respectively highly significant (p < .001) difference was observed similar to that seen on comparing the major study groups. \textbf{Thus there was no gender bias in APP values in diabetics with and without nephropathy. While in normal control group the mean values of APP were higher in males.} Similar higher values of APP have been found by Crook et al\textsuperscript{203} and M. Usman Khurshid et al\textsuperscript{576} in diabetic male and female patients compared to control subjects.

As per table 56 & figure 41 the mean values of TSA in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 72.33 ± 5.14 mg/dl, 74.02 ± 7.33 mg/dl, 73.92 ± 6.51 mg/dl & group C (T2DMN) were 79.59 ± 4.94 mg/dl, 83.01 ± 6.56 mg/dl, 82.40 ± 7.17 mg/dl as compared to control (group A) 61.03 ± 5.08 mg/dl, 61.43 ± 4.53 mg/dl, 59.56 ± 5.63 mg/dl respectively. The mean values of ceruloplasmin in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 35.10 ± 3.29 mg/dl, 35.76 ± 4.0 mg/dl, 35.56 ± 3.58 mg/dl & group C (T2DMN) were 41.00 ± 3.91 mg/dl, 44.80 ± 4.48 mg/dl, 43.95 ± 4.52 mg/dl as compared to control (group A) 25.56 ± 1.32 mg/dl, 26.79 ± 2.39 mg/dl, 26.75 ± 2.78 mg/dl respectively. The mean values of hsCRP in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 1.73 ± 0.75 mg/L, 2.16 ± 1.49 mg/L, 1.94 ± 0.86 mg/L &
group C (T2DMN) were 2.36 ± 1.43 mg/L, 3.35 ± 1.44 mg/L, 2.98 ± 1.40 mg/L as compared to control (group A) 0.89 ± 0.19 mg/L, 1.20 ± 0.50 mg/L, 1.19 ± 0.54 mg/L respectively.

The mean values of parameters of APP when compared statistically among control subjects of group A (table 57) in the age groups 21-40 yrs & 41-60 yrs and age groups 21-40 yrs & 61-80 yrs significantly higher mean values were seen in age group 41-60 yrs and 61-80 yrs as compared to 21-40 yrs (p ≤ .05) for Ceruloplasmin and hsCRP and not significant difference (p > .05) was seen for TSA in both the groups. Mojahedi MJ et al\textsuperscript{577} in their study found similar higher values of hsCRP with increasing age of diabetics and correlated it with increasing proteinuria. Whereas among the age group 41-60 yrs & 61–80 yrs no statistically significant difference was seen in mean values of TSA, Ceruloplasmin and hsCRP (p > .05). The mean values of parameters representing APP when compared statistically among subjects of the three subgroups (on the basis of Age) in group B, and group C the differences were not significant (p > .05) statistically (table 58 & 59). Thus the control group showed increasing values of APP with increasing age group, while the diabetics with and without nephropathy showed no such trend. Similar non significant difference in ceruloplasmin levels of diabetics in different age groups have been shown by Walter RM et al\textsuperscript{277}, Cunningham J et al\textsuperscript{278} and McMillan DE et al\textsuperscript{280}.Study by A. Merat et al\textsuperscript{568} show no difference in TSA value in different age groups in male diabetics, while the female diabetics showed increased value in postmenopausal age group.

When the mean values of parameters representing APP were compared statistically among the age group 21-40 yrs in group A & B significantly higher (p < .001) values were observed in diabetics (group B) compared to control subjects (group A). Among group A & C highly significant (p < .001) difference was observed for TSA and ceruloplasmin whereas significant (p ≤ .05) difference was observed for hsCRP. In group B & C
statistically significant \( (p \leq .05) \) difference was observed for TSA and ceruloplasmin with higher mean values in diabetics with nephropathy (group C) whereas not significant \( (p > .05) \) difference was observed for hsCRP (table 60).

When the mean values of parameters of APP were compared statistically in the age group 41-60 yrs and age group 61-80 yrs among groups A & B, groups A & C and group B & C highly significant \( (p < .001) \) difference was observed for all the parameters with higher values in diabetics and highest values in diabetics with nephropathy (group C) as compared to control subjects (group A) (table 61 & 62). This was in accordance with the findings in major study groups.

**OXIDANTS & ANTIOXIDANTS IN DIABETES**

Enzymatic antioxidants directly involved in the detoxification of ROS are SOD and hydroxyperoxidases such as catalase (CAT) and glutathione peroxidase (GSHPx). Apart from these endogenous antioxidants, an important source of antioxidants is in the diet, which contains numerous compounds exhibiting antioxidant activity. The most prominent dietary antioxidants are tocopherols, the fat-soluble vitamin (vitamin E), ascorbate water-soluble vitamin (vitamin C) and carotenoids. Subjects with diabetes may be especially prone to oxidative stress, which enhances the development and progression of diabetic microvascular and macrovascular complications.\(^{328, 329}\) Animal and human studies and in vitro experiments all suggest a role of oxidative stress, via an increased formation of free radicals in the pathophysiology of diabetic microvascular complications\(^{328, 329}\) such as nephropathy and retinopathy. Recent studies have reported a direct link between the imbalance of oxidative stress and antioxidants leading to impaired glucose uptake. In uncontrolled diabetes, the level of SOD, the enzyme responsible for inactivating the superoxide radical, along with the levels of the antioxidants vitamins are decreased.\(^{27}\) Ascorbate is a powerful reducing agent capable of rapidly
scavenging a number of ROS such as superoxide, H₂O₂ and singlet oxygen. Various studies have reported protective effects of antioxidants such as vitamin C¹⁴¹⁻¹⁴³ against oxidative damage of diabetes. The level of vitamin C in plasma and renal tissues is significantly reduced in diabetic patients.¹⁴², ¹⁴⁴ Impaired antioxidant function also plays a role in the development of diabetic kidney disease. Human studies with antioxidants for DN are limited and have had variable results.³⁹⁵

Table 63 & figure 42 show the mean values of MDA in diabetics group B (T2DM) 2.67 ± 0.69 nmol/ml, group C (T2DMN) 3.0 ± 0.81 nmol/ml as compared to control (group A) 1.77 ± 0.57 nmol/ml. Also the mean values of SOD & vitamin C in diabetics group B (T2DM) were 4.86 ± 0.65 U/ml 1.07 ± 0.10 mg/dl, Group C (T2DMN) were 4.47 ± 0.68 U/ml & 0.73 ± 0.12 mg/dl as compared to control (group A) 6.01 ± 0.87 U/ml & 1.42 ± 0.16 mg/dl. When the mean values of MDA, SOD and vitamin C were compared statistically in the three groups highly significant (p < .001) difference was observed for all with higher MDA levels and lower SOD and vitamin C levels in diabetics (table 64). Madhur M. Gupta et al²⁶, Vivian Samuel T³⁰, Jacek Rysz et al³⁸⁶, Aaseth J et al³⁹², Varvarovska J et al³⁹³ and Mukherjee B et al³⁹⁴ in their experimental and patients studies have shown similar results for MDA, SOD and vitamin C. Padayatty et al³⁹⁶, Punithavatki VR et al⁴⁰⁷, Je HD et al⁴⁰⁹ and Yıldırım O et al⁴¹⁰ got similar results for vitamin C in their experiments in diabetic rats and MM Suchitra et al⁵⁷⁸, Pasupathi P et al⁵⁷⁹ in type 2 diabetics. Fumiaki Kimura et al⁵⁸⁰ and SA Moussa et al⁵⁸¹ in their studies got raised SOD levels in diabetics and suggested that increase in serum SOD may reflect decrease binding of the enzyme to the endothelium, resulting in vascular wall being more vulnerable to oxidative damage.

Hyperglycemia is found to promote lipid peroxidation of low density lipoprotein (LDL) by a superoxide-dependent pathway resulting in the generation of free radicals.³³², ³³³ ROS degrade polyunsaturated lipids, forming MDA.³⁵⁶ This compound is a reactive aldehyde and is one of
the many reactive electrophile species that cause toxic stress in cells and form covalent protein adducts referred to as advanced lipoxidation end-products (ALE), in analogy to AGE.\textsuperscript{357} Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation. A rise in plasma MDA levels in the later stage of DM reflects oxidative damage to lipids. Several studies, including human\textsuperscript{392,393} and experimental\textsuperscript{394} have reported significant depletion of GSHPx in diabetics associated with enhanced lipid peroxidation as a result of decreased scavenging of free radicals. In diabetic patients, the autoxidation of glucose results in the formation of hydrogen peroxide which inactivates SOD. Therefore, the accumulation of hydrogen peroxide may be one of the explanations for decreased activity of SOD in these patients. The primary catalytic cellular defense that protects cells and tissues against potentially destructive reactions of superoxide radicals and their derivatives is the Cu/Zn-SOD. It has been observed that SOD can be rapidly induced in some conditions when cells or organisms are exposed to oxidative stress. The low activity of SOD in our study in type II diabetes may suggest that with longer disease duration, SOD induction and consequently its activity progressively decrease, since nonenzymatic glycation, the other cause of hydrogen peroxide production, later predominates and further inhibition of Cu/Zn SOD occurs. A likely explanation for a lower vitamin C status in diabetics is that ascorbic acid is actively transported into the cells in its partially oxidized form as dehydroascorbic acid, which is promptly converted to ascorbic acid within the cell. The carrier of ascorbic acid transport serves also to transport glucose and is inhibited in transporting ascorbic acid by the hyperglycemia of diabetics. Decrease in vitamin-C levels could be due to its increased utilization in the antioxidant defense against elevated lipid peroxidation due to oxidative stress.\textsuperscript{582}

Table 65 & figure 43 show the mean values of MDA in diabetics with microalbuminuria (group C I) $2.72 \pm 0.60 \, \text{nmol/ml}$ & in diabetics with
macroalbuminuria (group C II) $4.21 \pm 0.33$ nmol/ml. Whereas the mean values of SOD & vitamin C in diabetics with microalbuminuria (group C I) were $4.51 \pm 0.68$ U/ml & $0.78 \pm 0.09$ mg/dl & in diabetics with macroalbuminuria (group C II) were $4.31 \pm 0.67$ U/ml & $0.56 \pm 0.07$ mg/dl respectively. The above data shows that the mean value of MDA in diabetics with macroalbuminuria (Group C II) was high as compared to diabetics with microalbuminuria (Group C I) & the difference was highly significant statistically ($p < .001$). SOD was low but the difference was not significant statistically ($p > .05$). Whereas the mean value of vitamin C was significantly lower in diabetics with macroalbuminuria as compared to diabetics with microalbuminuria ($p<0.001$). Similar findings of MDA, SOD & vitamin C have been seen in studies by Madhur M. Gupta et al, Jyoti Dwivedi et al, Vivian Samuel T & Ranjini KS et al in type 2 DM with retinopathy and nephropathy. The oxidative stress in DM is greatly increased due to prolonged exposure to glycemia and impairment of the oxidant/antioxidant balance. Lipids are among the primary targets of oxidative stress. Lipid peroxidation of the cellular structures, a consequence of increased oxygen free radicals, is thought to play an important role in atherosclerosis and microvascular complications of DM. Although microvascular and macrovascular complications of DM are known to increase with DM duration, the association between DM duration and MDA levels in type II DM remains controversial.

As per the findings of table 66 & figure 44 the mean values of MDA in male & female subjects of group B (T2DM) were $2.71 \pm 0.70$ nmol/ml, $2.64 \pm 0.67$ nmol/ml & group C (T2DMN) were $3.04 \pm 0.82$ nmol/ml, $2.94 + 0.81$ nmol/ml as compared to control (group A) $1.92 + 0.54$ nmol/ml, $1.61 + 0.56$ nmol/ml respectively. The mean SOD values in male & female subjects of group B (T2DM) were $4.83 + 0.59$ U/ml, $4.90 + 0.70$ U/ml & group C (T2DMN) were $4.46 + 0.69$ U/ml, $4.49 + 0.66$ U/ml as compared to control (group A) $6.02 + 0.85$ U/ml, $6.0 + 0.89$ U/ml respectively.
Vitamin C mean values in male & female subjects of group B (T2DM) were 1.06 + 0.10 mg/dl, 1.08 + 0.10 mg/dl & Group C (T2DMN) were 0.73 + 0.12 mg/dl, 0.74 + 0.11 mg/dl as compared to control (group A) 1.37 + 0.16 mg/dl, 1.47 + 0.15 mg/dl respectively. The mean values of MDA and vitamin C when compared statistically among both the male & female subjects in group A showed highly significant (p < .001) difference whereas SOD was not significant (p > .05). In group B and group C for all the parameters the difference was not significant statistically (p > .05) (table 67). Thus the control subjects showed higher mean value of MDA and lower mean value of vitamin C in males as compared to females. The diabetics showed no gender bias for all three parameter values when compared in the same group. Similar results have been put forth by Mallick et al584 and Abdoljalal Marjani et al585 for MDA and PJ Hisalkar et al586 for SOD in their gender wise study of oxidative stress in type 2 DM. While study by Yazum Bhutia et al587 and Soliman GZ et al366 have shown significantly higher values of MDA and SOD in male diabetics compared to female diabetics.

When the mean values of MDA, SOD & vitamin C were compared statistically among the male subjects and female subjects in groups A & B and groups A & C respectively, highly significant (p < .001) difference was observed. When group B & C were compared statistically highly significant (p < .001) difference was observed for SOD and vitamin C whereas significant (p ≤ .05) difference was observed for MDA (Table 68 & 69). These findings are in accordance with the comparative study of major groups (A, B & C). Similar findings were seen by Soliman GZ et al366, Palanduz S et al588, Bhatia S et al589, Maritim AC et al137, Sailaja YR et al590 and Onyesom I et al591 in their studies in type 2 DM for MDA, SOD and vitamin C.

The mean values of MDA in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 2.52 ± 0.50 nmol/ml, 2.70 ± 0.73 nmol/ml.
nmol/ml, 1.94 ± 0.86 nmol/ml & group C (T2DMN) were 2.57 ± 0.72 nmol/ml, 3.08 ± 0.82 nmol/ml, 2.87 ± 0.75 nmol/ml as compared to control (group A) 1.44 ± 0.27 nmol/ml, 1.79 ± 0.56 nmol/ml, 1.84 ± 0.65 nmol/ml respectively. The mean values of SOD in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 4.85 ± 0.73 U/ml, 4.87 ± 0.65 U/ml, 4.84 ± 0.61 U/ml & group C (T2DMN) were 4.72 ± 0.83 U/ml, 4.42 ± 0.67 U/ml, 4.54 ± 0.66 U/ml as compared to control (group A) 5.87 ± 1.00 U/ml, 6.08 ± 0.84 U/ml, 5.90 ± 0.87 U/ml respectively. The mean values of vitamin C in the age group 21-40 yrs, 41-60 yrs, 61-80 yrs of the subjects of group B (T2DM) were 1.09 ± 0.07 mg/dl, 1.07 ± 0.11 mg/dl, 1.08 ± 0.09 mg/dl & group C (T2DMN) were 0.81 ± 0.09 mg/dl, 0.72 ± 0.12 mg/dl, 0.75 ± 0.11 mg/dl as compared to control (group A) 1.53 ± 0.06 mg/dl, 1.41 ± 0.16 mg/dl, 1.42 ± 0.19 mg/dl respectively (table 70 & figure 45). The mean values of MDA and vitamin C when compared statistically among control subjects (group A) in the age group 21-40 yrs & 41-60 yrs and age groups 21-40 yrs & 61-80 yrs the difference were statistically highly significant (p ≤ .001) whereas the difference in SOD mean values was not significant (p > .05). Whereas in the age group 41-60 yrs & 61–80 yrs the difference in mean values of MDA, SOD and Vitamin C were all not significant (p >.05) statistically (table 71). Thus in control subjects the value of MDA increased with increasing age, the value of vitamin C decreased with increasing age and there was no significant change in value of SOD with increase in the age of subjects. Study by Ligia J. Dominguez et al592 in type 2 DM showed increased value of MDA and ROS in control subjects with increasing age.

The mean values of MDA, SOD and vitamin C when compared statistically among subjects of the three subgroups (on the basis of age) in diabetics without nephropathy (group B) & with nephropathy (group C) showed no statistical significant difference (p > .05) (table 72) except for vitamin C which was significantly higher (p < .05) in age group 21- 40 yrs as compared to 41-60 yrs age group among group C patients.
The non significant difference in MDA & SOD values may be explained by the fact that the duration of diabetes is the major factor deciding the oxidative stress rather than the age of the patient. This hypothesis has been supported by studies of Ligia J. Dominguez et al\textsuperscript{592}, Pasaoglu H et al\textsuperscript{593} and Nakhjavani M et al\textsuperscript{28} for MDA, they also showed that levels of SOD do not differ with age of patient and duration of diabetes. Kanti Bhooshan Pandey et al\textsuperscript{594} in their study of type 2 DM observed significantly ($p < 0.05$) higher erythrocyte MDA level in old and middle age group diabetic patients compared to sex and age matched healthy subjects, however no significant elevation was measured in young group subjects. Vitamin C is exogenous antioxidant and its level depends upon dietary intake, which may be the reason for higher vitamin C levels in younger patients compared to the elderly.

When the mean values of MDA, SOD and vitamin C were compared statistically among the age group 21-40 yrs in group A & B and group A & C – the difference in mean values of MDA and vitamin C were highly significant ($p < .001$) and SOD was significant statistically ($p \leq .05$). While in group B & C the difference in the mean values of all the parameters were not significant ($p > .05$) (table 74). When the mean values of MDA, SOD and vitamin C were compared statistically in the age group 41-60 yrs and 61-80 yrs respectively among the group A & B, group A & C and group B & C the difference observed was highly significant ($p < .001$)(table 75), except for significant ($p \leq .05$) difference was observed for SOD and not significant ($p > .05$) difference was observed for MDA in the age group 61-80 yrs among group B & C(table 76). Thus the comparison of MDA, SOD and vitamin C in groups A, B & C based on subgroups as per age showed similar trend as that of the major study groups’ comparison with increasing values of MDA in diabetics and decreasing values of SOD & vitamin C with lowest in diabetic nephropathy patients.
The results suggest that the increase in lipid peroxidation and the decline in antioxidant defenses may appear early in type 2 DM patients, before the development of secondary complications, and might play an important role in the initiation and progression of diabetic complications. Our results also suggest that there seems to be an imbalance between plasma oxidant and antioxidant systems in patients with type 2 diabetes.

Correlation between biochemical parameters among the three groups of patients: (Table 77-82)

Hyperglycemia in type 2 diabetes is associated positively with renal parameters, acute phase proteins, dyslipidemia, oxidants and negatively associated with antioxidants. The possible reasoning’s and hypotheses have been discussed above. Thus these parameters on applying Pearson correlations showed correlation with each other in control and type 2 diabetes with and without nephropathy as follows-

1. The fasting and post prandial glucose levels showed highly significant and positive correlation in all 3 study groups A (control); B (T2DM) & C (T2DMN) with each other and with HbA1c. Similar positive correlation of HbA1c with blood glucose levels in type II DM has been seen in studies by Trivelli LA et al, Gabbay KH et al, Gonen B et al, Koenig RJ et al and Nathan DM et al. At the present time, HbA1c is used worldwide as the marker of long term glycaemic control and also a therapeutic target in the prevention and delay of the development of hyperglycaemic complications. Genuth S et al and Edelman D et al in their studies concluded that HbA1c remains a very useful test in the measurement of glycaemic control. Engelgau MM et al, ADA and DCCT studies pointed out that HbA1c though not always reliable in every patient, is by far the most universally accepted method of glycaemia measurement in clinical practice today.
The glycemic parameters (FBS, PPBS and HbA1c) showed significant positive correlation in type II diabetics (group B&C) with dyslipidemia, mainly triglycerides and VLDL levels. HbA1c showed direct and significant correlations with cholesterol, triglycerides and LDL and inverse correlation with HDL in diabetic patients with nephropathy compared to without nephropathy in studies by various authors - Syed Muhammad Shahid et al123, Haseeb Ahmad Khan et al124, M. Rema et al125, Framingham Heart Study295 and Arshag D Mooradian et al.562 Study by H.A. Elnasri et al563 indicates a positive association between age and lipid abnormalities, with higher levels among older than younger ages. The prevalent combination of lipid abnormalities was that of elevated TG and reduced HDL. Anthonia O Ogbera et al564 and Attman, PO et al307 in their studies in diabetic nephropathy (DN) showed positive association of glycemic parameters with an altered lipid profile characterised by elevated triglyceride and VLDL. Garry X Shen16 showed positive correlation of triglycerides and negative correlation of HDL with blood glucose levels in diabetics. LDL cholesterol was the strongest independent predictor of CHD followed by HDL cholesterol in UKPDS study.114 The results of studies by B Shivananda Nayak et al569 and M. Usman Khurshid et al576 indicate a clear association between glycaemic control of diabetes and appearance of dyslipidaemia. There was a statistically significant association of triglycerides and HDL-C with increasing age, female sex, obesity, physical inactivity and poor glycaemic control of diabetes.

Significant positive correlation of glycemic parameters (FBS, PPBS and HbA1c) was seen in type II diabetics (group B&C) with acute phase proteins- hs-CRP, total sialic acid and ceruloplasmin in present study. Hyperglycemia is an associated factor to the increase of serum CRP levels, in uncontrolled type II diabetic subjects. Soinio, M et al215, Martha RM et al217, Jager A et al219, Pfutzner A et al220 and Safiullah Amanullah et al222 showed that HbA1c significantly correlates with hs-CRP levels in type I and type II DM. Studies by Crook MA et al203 and Evans T
C et al\textsuperscript{153} showed positive correlation of Sialic acid with HbA\textsubscript{1c}. Pfutzner A et al\textsuperscript{220} and Masuda et al\textsuperscript{253} have shown that serum TSA reflects the status of blood glucose control and the progression of ischemic disease of the lower extremities in type II DM. Zahedi et al\textsuperscript{254} have found that TSA increased post prandially with positive correlation to glucose levels, giving further insight as to why it is considered to be a cardiovascular risk factor. 

**Serum TSA is a newly established potential risk factor for the development of macro and microvascular complications of diabetes.** SA plays an important role in the pathophysiology of endothelial dysfunction and vascular disease in DM, and in part explain the increased levels of SA in diabetic nephropathy with positive correlation with glycemic status as seen in studies by Syed M. Shahid et al\textsuperscript{189} and Pickup JC et al\textsuperscript{190} A. Melidonis et al\textsuperscript{236}, Wakabayashi I et al\textsuperscript{255}, Syed Muhammad Shahid et al\textsuperscript{252} and A. Merat et al\textsuperscript{568} studied SA in DM retinopathy and showed positive correlation with HbA\textsubscript{1c}. The uniform increase in fasting plasma glucose, glycosylated hemoglobin (HbA\textsubscript{1c}), serum fructosamine, glycosylated plasma protein, serum hexosamine and serum sialic acid levels in diabetic patients indicates that the process of glycosylation depends upon hyperglycemia.\textsuperscript{569} An increase in serum ceruloplasmin levels has also been reported in type II DM by Walter R M et al\textsuperscript{277}, Cunningham J et al\textsuperscript{278} and McMillan DE et al.\textsuperscript{280} Cross-sectional studies in newly diagnosed or established type II diabetic patients by Temelkova-Kurktschiev T et al\textsuperscript{182}, Rodríguez-Mora’n M et al\textsuperscript{184}, Arnalich F et al\textsuperscript{185} and Leinonen E et al\textsuperscript{186} in relation to APP & oxidative stress - have confirmed that inflammatory and acute-phase markers such as CRP, IL-6, TNF-\textalpha, nitric oxide, ceruloplasmin, ferritin, sialic acid and others are elevated in these subjects compared with nondiabetic control subjects and show positive correlation with glycemic parameters. However, it has been reported that blood HbA\textsubscript{1c} levels, duration of type II DM, patient age, and the presence or absence of diabetes complications are not major factors influencing the ceruloplasmin
HbA\textsubscript{1c} also showed strong positive correlation with microalbuminuria in diabetics in present study. Similar positive correlation has been seen in studies by Stratton IM et al\textsuperscript{154}, Bahman P T et al\textsuperscript{538}, Rossing, P et al\textsuperscript{423}, Klein R et al\textsuperscript{435} and Massoud Amini et al\textsuperscript{553}.

**Significant positive correlation of glycemic parameters (FBS, PPBS and HbA\textsubscript{1c}) was seen in type II diabetics (group B&C) with MDA.** Highly significant negative correlation of glycemic parameters was seen with vitamin C and SOD in all three groups of patients. Positive correlation between the MDA level and indices of glycemic control in type II DM with increased levels of serum MDA in cases of diabetes mellitus with complications indicating positive association of oxidative stress with diabetic complications have been found in studies by Vivian Samuel T et al\textsuperscript{30}, Fadupin GT et al\textsuperscript{413} and Harding AH et al\textsuperscript{417} showed negative correlation of vitamin C with glucose levels in type II DM. Madhur M. Gupta et al\textsuperscript{26} showed negative correlation of SOD with hyperglycemia in diabetics with and without retinopathy. Jyoti Dwivedi et al\textsuperscript{29}, Fumiaki Kimura et al\textsuperscript{580} and Vivian Samuel T et al\textsuperscript{30} observed a strong negative relationship between the serum concentration of extra cellular SOD and the severity of both micro- and macrovascular diabetic complications and the lowest SOD activities were noticed in patients with poor diabetic control.

**FBS, PPBS and HbA1c showed highly significant positive correlation with microalbuminuria in all the three study groups.** Positive correlation of HbA\textsubscript{1c} with diabetic nephropathy (microalbuminuria) has been shown in studies done by DCCT\textsuperscript{113}, Koenig RJ et al\textsuperscript{338}, ADA\textsuperscript{120} and Shehnaz A Sheikh et al.\textsuperscript{11} In these studies tight glycemic control has been shown to positively influence the risk outcomes in diabetic nephropathy. On a cellular level, it has been known to reverse glomerular hypertrophy and hyperfiltration, both important mechanisms for early glomerular injury (Mogensen, CE et al\textsuperscript{456}). Clinically, this has been shown following the results of the Diabetes Control and Complications Trial (DCCT)\textsuperscript{113} and the
United Kingdom Prospective Diabetes Study (UKPDS).\textsuperscript{114,425} They have evaluated the relationship between glycaemic control and nephropathy, retinopathy and neuropathy. Araki S et al\textsuperscript{445} noted that lower values of HbA\textsubscript{1c} of < 6.95\% were independently associated with regression and remission of microalbuminuria in patients over a 6 year period. Clinical and epidemiological data from human studies suggest that the magnitude and duration of hyperglycemia in diabetes are strongly associated with the severity of microvascular complications (Engerman RL et al\textsuperscript{438} and Danne T et al\textsuperscript{439}).

Thus increased blood glucose levels showed parallel increase in HbA\textsubscript{1c}, microalbuminuria, total sialic acid, MDA, ceruloplasmin, hs-CRP, triglycerides and VLDL. While increase in glucose levels showed corresponding decrease in vitamin C and SOD levels. These observations suggest a role of hyperglycemia in acute phase reaction, oxidative stress and decreased antioxidants in diabetics.

2. Blood urea and creatinine showed strong positive correlation with each other in all the 3 groups, while highly significant positive correlation of urea and creatinine was seen with ceruloplasmin, hs-CRP, MDA and, significant positive correlation with microalbuminuria and total sialic acid in diabetics with nephropathy (gr. C). Studies by Tejal J Wagle et al\textsuperscript{554}, Adler Al et al\textsuperscript{444}, Emoto M et al\textsuperscript{548} Mittal A et al\textsuperscript{543}, Gurjeet Singh et al\textsuperscript{544} and Anupriya Sharma et al\textsuperscript{547} showed positive correlation of urea and creatinine with micral and HbA\textsubscript{1c}. Qin LX et al\textsuperscript{595} and Vadde Ramakrishna et al\textsuperscript{149} showed positive correlation of urea and creatinine with serum and urinary ceruloplasmin in type 2 diabetics and type 1 diabetics respectively. Study by Anubha Mahajan et al\textsuperscript{20} showed positive correlation of urea and creatinine with hs-CRP in type 2 diabetics. Study by Shehnaz A Sheikh et al\textsuperscript{11}, UKPDS\textsuperscript{444}, Rossing, P et al\textsuperscript{423} and Mittal A et al\textsuperscript{543} has found positive correlation of microalbuminuria with serum creatinine levels and progression of diabetic nephropathy. Serum sialic acid, glucose and
HbA1c showed positive correlation with urea and creatinine levels in patients with diabetic nephropathy compared to diabetic patients without nephropathy in study by Syed Muhammad Shahid et al.\textsuperscript{189, 252} Strong negative correlation of urea and creatinine was seen with vitamin C in group C. Study by Madhur M. Gupta et al\textsuperscript{26} showed negative correlation of vitamin C and SOD with progression of diabetes. Study by Jyoti Dwivedi et al\textsuperscript{29} showed positive correlation of MDA and negative correlation of vitamin C with diabetic nephropathy.

3. **Microalbuminuria showed highly significant positive correlation with UACR, total sialic acid, ceruloplasmin, hs-CRP and MDA in all the 3 groups.** Similarly serum MDA concentration was significantly higher in diabetic nephropathy while catalase & SOD activity in group of diabetic nephropathy was significantly lower than group without diabetic nephropathy in studies by Jyoti Dwivedi et al\textsuperscript{29} and Nakhjavani M et al\textsuperscript{28}. Hyperglycemia has been shown as an associated factor to the increase of serum CRP levels, in uncontrolled type II diabetic subjects with microalbuminuria in studies by Soinio M et al\textsuperscript{215}, Martha RM et al\textsuperscript{217}, Jager A et al\textsuperscript{219}, Pfutzner A et al\textsuperscript{220} and Safiullah Amanullah et al\textsuperscript{222}. Studies by Mogensen, CE et al\textsuperscript{463}, Zelmanovitz T et al\textsuperscript{497} and Nathan DM et al\textsuperscript{513} in type II DM found an equally high correlation between 24-hour urine albumin excretion and both the urinary albumin concentration and the ACR in the first morning urine. There is a significant positive correlation between microalbumin excretion and sialic acid in type II DM as shown in studies by Krishnamurthy U et al\textsuperscript{571} and Shivananda Nayak B et al\textsuperscript{572}. Diabetic patients with nephropathy showed more significant positive correlation of plasma ceruloplasmin with microalbuminuria compared to diabetics without nephropathy in study by Bogdana Vîrgolici et al\textsuperscript{131}.

**Significant positive correlation of microalbuminuria was seen with cholesterol, triglycerides, LDL, VLDL, LDL: HDL and duration from**
onset of diabetes in diabetics with nephropathy (gr. C). Evans T C et al, Mittal A et al, Gall MA et al, Massoud Amini et al, and Muhammad Yakoob Ahmedani et al in their study found positive correlation of dyslipidemia like increased triglycerides, LDL and lowered HDL with microalbuminuria in type II DM. **Highly significant negative correlation of microalbuminuria levels was seen with vitamin C and SOD in all the three groups of patients.** Similar findings have been seen in studies by Madhur M. Gupta et al and Jyoti Dwivedi et al.

4. Cholesterol showed highly significant positive correlation with triglycerides, HDL, LDL, VLDL, Chol:HDL and LDL:HDL in all the 3 groups and with sialic acid in group C. Significant positive correlation was seen of cholesterol with UACR, MDA and hs-CRP in group C. Triglycerides showed strong positive correlation with other parameters of lipid profile in all the 3 groups. Highly significant positive correlation of triglycerides was seen with sialic acid, ceruloplasmin, MDA and hs-CRP in group C. Significantly elevated serum TSA in the severely hypertriglyceridaemic group of type II DM has been shown in studies by B Shivananda Nayak et al and M. Usman Khurshid et al. Also, a good correlation was observed between sialic acid and important cardiovascular risk factors such as cholesterol, LDL and TG in study by Shivananda Nayak B et al. Cook CB et al and Onovughakpo-Sakpa O et al in their study showed positive correlation of lipid parameters with UACR in diabetics with CVD. Study by Anubha Mahajan et al and Nakano S et al showed positive correlation of cholesterol, LDL and triglycerides with hs-CRP in type 2 diabetics. Altomare E et al in their study found positive correlation of triglycerides with MDA levels in poorly controlled type 2 diabetes. Memişoğlu Re et al in their study found positive correlation of cholesterol and triglycerides with CRP, MDA and ceruloplasmin in type 2 diabetic patients.
Significant negative correlation of triglycerides with SOD & vitamin C was seen in groups B&C. Study by Mosaad A et al showed positive correlation of MDA and negative correlation of SOD with cholesterol and triglyceride levels in type 2 diabetes. HDL showed significant positive correlation with cholesterol and LDL in all the 3 groups and highly significant negative correlation was seen with chol:HDL and LDL:HDL in group B & C. LDL showed strong positive correlation with all lipid profile parameters in all the 3 groups while significant positive correlation was seen with sialic acid in group C. VLDL showed highly significant positive correlation with lipid profile parameters in group B & C and with sialic acid, ceruloplasmin, MDA and hs-CRP in group C. VLDL showed significant negative correlation with SOD and vitamin C in groups B&C.

Thus the values of triglycerides and VLDL were higher in diabetics with higher values of FBS, PPBS, HbA1c, microalbuminuria, ceruloplasmin, sialic acid, MDA and hs-CRP.

5. Total sialic acid and ceruloplasmin showed strong positive correlation with each other, MDA, and hs-CRP in all three groups. Highly significant positive correlation of MDA and hs-CRP was seen with each other in all the 3 groups, while positive correlation was seen with UACR in group B & C. Total sialic acid, hs-CRP, MDA and ceruloplasmin showed highly significant negative correlation with SOD and vitamin C in diabetics with and without nephropathy. Madhur M. Gupta et al, Vivian Samuel T, Jacek Rysz et al, Aaseth J et al, Varvarovska J et al and Mukherjee B et al in their experimental and patients studies have shown similar results for MDA, SOD and vitamin C. J Chen et al showed positive correlation of sialic acid with microalbuminuria and UACR in type 2 diabetics with and without nephropathy. Study by Vishakha V Mahajan et al showed
positive correlation of TSA, ceruloplasmin and CRP with each other and with LDL:HDL cholesterol ratio and microalbuminuria in type 2 diabetics.

6. On comparing the duration of diabetes and various biochemical parameter levels, highly significant positive correlation was seen with sialic acid and significant positive correlation was seen with microalbuminuria, ceruloplasmin and hs-CRP. Vishakha V Mahajan et al\textsuperscript{199} showed similar correlation of duration of diabetes with sialic acid, ceruloplasmin and CRP. The development and progression of chronic complications in type II DM are been related to longer duration of diabetes in studies by Hashim R et al\textsuperscript{428}, Parving H et al\textsuperscript{443}, Niskanen LK et al\textsuperscript{551} and Bahman P T et al\textsuperscript{538}. Study by Shehnaz A Sheikh et al\textsuperscript{111} showed Microalbuminuria had a highly significant correlation with duration of diabetes.